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**PURIFICATION PROCESS FOR MANUFACTURING A ~~HIGH~~ HIGHLY
PURE ACARBOSE**

Background of the Invention

1. Filed— Field of the Invention

The present invention relates to a process for manufacturing ~~high~~ a highly
pure acarbose, ~~more particularly, and~~ particularly to a process which uses
5 alcohol for precipitation and separation, and a strongly-strong cation exchange
chromatography and an immobilized enzyme affinity chromatography for
manufacturing a ~~high-pure~~ highly pure acarbose to treat diabetes.

2. Description of the Related Art

10 Acarbose,

O-4,6-Dideoxy-4-[[[1S-(1 α ,4 α ,5 α ,6 α)]-4,5,6,-trihydroxy-3-(hydroxymethyl)-2-c
yclohexen-1-yl]amino]- α -D-glucopyranosyl-(1 \rightarrow 4)-O- α -D-glucopyranosyl-(1 \rightarrow
4)-D-glucose, C₂₅H₄₃NO₁₈, Mw 645.63, it is an oligo-~~derivatives~~ derivative. The
~~acarbose~~ Acarbose inhibits the activity of α -glucosidase at the edge ~~in~~ of the
15 small intestine by invertibility for slowly turning complex carbohydrates
~~complex~~ and disaccharide into glucose, which can be absorbed by human

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humans, to decrease concentration of triglycerol and insulin in blood and blood sugar.

In the early 1970s, ~~the~~ acarbose could improve the ratio of meat and fat, so it ~~uses~~ was used to add as an additive in the feed to feed the for animals such as ~~pigs~~. Recently, ~~the researches~~ researchers have found that find the acarbose could ~~control~~ controls the blood sugar of NIDDM and decreases the insulin value after ~~diet eating~~, for preventing diabetic cardiovascular ~~complication~~ complications, but it. However, acarbose cannot ~~can~~ not directly change the insulin resistance. ~~The acarbose~~ Acarbose only has a few aftereffects, such as abdominal distension, borborygmus and diarrhea, ~~being~~ which go away after ~~treating a period of treatment~~, and it ~~hardly affects~~ affect the health. The glucobay of the Bayer ~~is~~ was first approved ~~first~~ in 1995 by FDA. So far, the acarbose is primarily manufactured using manufacturing acarbose is mainly used *Actinoplanes sp.* or *Streptomyces glaucescens*.

The DOH of Taiwan adjusts the adjusting blood sugar material to be health food. Further, ~~the fat reason of the Easterner~~ Easterners is eating eats polysaccharide which differs ~~different from the westerner is eating fat eaten by~~ Westerners. Therefore, ~~the~~ acarbose not only treats diabetes, but can also ~~uses~~ be used in the diet food.

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US. Pat. 4,062,950 ~~of sets forth a~~ recover and purification process for manufacturing acarbose, and discloses that acarbose-containing fermentation broth is discolored by anion resins or activated carbons in the ~~acid~~ acidic condition, and that acarbose are absorbed by activated carbons in the neutral condition and are eluted by ethyl alcohol solution or acetone solution in the ~~acid~~ acidic condition. The elute passes through the cation exchange chromatography, and acarbose are finally washed by the acid or base solution. The eluted liquid is counteracted and concentrated in the vacuum, and ~~the 85% purity of the~~ acarbose with 85% purity is precipitated by the organic solvent. The high purity of the acarbose can be manufactured if the exchange chromatography uses celluloses ~~to be as~~ as a matrix. Further, the liquid is concentrated and precipitated by the organic solvent to get a ~~high purity of the~~ highly pure acarbose. The process is ~~complicate~~ complicated because the process must use the activated carbons for absorbing and the exchange chromatography process repeated many times for purification of acarbose.

US. Pat. 4,174,439 mixes cation and anion exchange resin into acarbose-containing fermentation broth to absorb acarbose and elutes the acarbose by deionized water. The acarbose solution ~~process is~~ processed twice by a cation and anion exchange resin and is eluted by hydrochloric acid, and

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~~process~~ is processed with a neutralizing treatment by an anion exchange resin and frozen until dry to get 52%-58% purity of the acarbose with 52-58% purity.

Further, US. Pat. 4,666,776 and US. Pat. 4,767,850 improve US. Pat. 4,174,439 to use ~~strongly cation~~ strong cation exchange resin ~~and~~, be washed by hydrochloric acid, ~~and be processed by a process-neutralizing treatment by with~~ an anion exchange resin and frozen until dry to get 79%-82% purity of the acarbose with 79-82% purity.

The above ~~mentions methods~~ of purifying the acarbose ~~process~~ all repeat the anion and cation exchange chromatography to get the acarbose solution and finally use cation exchange chromatography to get a high concentration of acarbose. ~~But~~ However, it is difficult to achieve a the purity of acarbose is hard to be sufficient for use as a medical drug.

US. Pat. 4,904,769 discloses a method in which ~~taking~~ impure acarbose passes through a weakly-weak cation exchange chromatography containing carbonyl, cellulose, and dextran in ~~with~~ specific temperature and pH ~~value~~ values to get 90% purity acarbose with a 90% purity. The process is complicated and uses ~~weakly-weak~~ ion exchange chromatography ~~being in~~ an expansive process, resulting in high manufacturing ~~costs~~ costs.

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5 ~~And Finally~~, WO: 99/07720 discloses ~~which~~ taking an impure acarbose ~~manufacturing~~ manufactured by US. Pat. 4,174,439, US. Pat. 4,666,776 and US. Pat. 4,767,850, ~~which~~ passes through a ~~strongly cation~~ strong cation exchange chromatography containing non-aromatic to get ~~high pure~~ highly pure acarbose, and the processes are typically ~~has~~ complicated ~~process~~ and ~~with~~ high manufacturing ~~cost~~ costs.

Summary of the Invention

10 ~~According to~~ As discussed above ~~mention~~, the present invention improves the complicated process and high manufacturing ~~cost~~ costs of the prior art, and ~~get~~ achieves a ~~high pure~~ highly pure acarbose ~~being~~ appropriate for use as a medical drug.

15 The present invention considers processes and material of the above ~~mentions~~ mentioned prior art, to improve an impure acarbose manufacturing process which applies the solubility between the acarbose and alcohol or methyl alcohol ~~and~~, absorbs the acarbose by ~~strongly~~ strong exchange resin, and eliminates like acarbose by sodium chloride and ammonia solution. The ~~75%-80% purity~~ 75-80% purity ~~could get~~ can be achieved by eluting high concentration ammonia solution, and finally ~~passes~~ passing through

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an α -glucosidase column to get up 95% purity pure acarbose, to overcome thereby overcoming the high manufacturing cost and costs complicated processes of the prior art.

Further scope of the applicability of the present invention will become
5 apparent from the detailed description given hereinafter. However, it should be
understood that the detailed description and specific examples, while indicating
preferred embodiments of the invention, are given by way of illustration only,
since various changes and modifications within the spirit and scope of the
invention will become apparent to those skilled in the art from this detailed
10 description.

Brief Description of the Drawings

~~The accompanying drawing is included to provide a further understanding~~
~~of the invention, and is incorporated in and constitutes a part of this~~
15 ~~specification. The drawing illustrates an embodiment of the invention and,~~
~~together with the description, serves to explain the principles of the invention.~~
~~In the drawing, The present invention will become more fully understood from~~
the detailed description given hereinbelow and the accompanying drawings

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which are given by way of illustration only, and thus are not limitative of the present invention, and wherein:

Fig. 1 is a flow chart showing a purification process for manufacturing a ~~high-pure~~highly pure acarbose of the present invention;

5 Fig. 2 is flow chart showing a purification process for manufacturing a ~~high-pure~~highly pure acarbose of Example 1 of the present invention;

Fig. 3 is flow chart showing a purification process for manufacturing a ~~high-pure~~highly pure acarbose of Example 2 of the present invention;

10 Fig. 4 is flow chart showing a purification process for manufacturing a ~~high-pure~~highly pure acarbose of Example 3 of the present invention; and

Fig. 5 is flow chart showing a purification process for manufacturing a ~~high-pure~~highly pure acarbose of Example 4 of the present invention.

Detailed Description of the preferred Embodiments

15 Refer to Fig. 1, the present invention discloses a purification process for manufacturing ~~high-pure~~highly pure acarbose ~~comprises-comprising~~ the steps of:

~~step-Step~~ 10, start;

~~step-Step~~ 15, adding alcohol in an acarbose-containing fermentation broth for precipitation;

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~~step~~ Step 20, passing sediments through ~~strongly cation~~ strong cation exchange resin and processing an immobilized enzyme affinity chromatography process.

The present invention discloses a ~~purification and process for purifying~~
5 ~~acarbose process from acarbose containing~~ acarbose-containing fermentation broth to get a ~~high pure~~ highly pure acarbose to treat diabetes. The ~~strongly cation~~ strong cation exchange chromatography uses styrene divinylbenzene copolymer without methoxymethylmethacrylamide to be a resin matrix, and the enzyme of the immobilized enzyme affinity chromatography uses
10 α -amyloglucosidase(α -glucoamylase).

Further, an upper liquid of the acarbose-containing fermentation broth is made by centrifugal effect or filter and concentrates 1/10 volume by a rotary
~~evaporators~~ evaporator concentrating system. Then, adding adequate ethyl alcohol solution or methyl alcohol solution takes an upper liquid by
15 centrifugating, and the upper liquid ~~concentrates to consistency~~ forms a concentrate. Finally, the ~~consistency concentrate~~ uses ethyl alcohol to get a sediment of the ~~containing~~ acarbose, and the sediment is ~~solved~~ dissolved by distilled water to be in a 200 mg/mL concentration ~~and~~. The pH of the

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dissolved sediment is adjusted to a level of approximately 5-9, adjusts pH-5-9
to be a mixing liquid.

The process of ion exchange resin ~~describes using strongly cation~~ uses a
strong cation exchange resin, such as AMBERJET 1200 H resin or AMBERJET
5 1200 Na (Rohm and Hass Company), and is washed by deionized water ~~till until~~
the pH value of ~~an the~~ upper liquid is ~~large larger~~ than 4. Then, ~~taking the~~
strong cation exchange resin containing 20-200 mg sugar/mL ~~adds is added~~ into
the mixing liquid and blending blended for 10-30 minutes, ~~and taking a~~. A part
of the resin is then washed several times ~~by with~~ distilled water. The resin is
10 then washed by NaCl to get obtain a lot of acarbose-like sugars, and is eluted by
an 0.75N ammonia solution. Finally, the resin is ~~solved dissolved~~ by a 1.5N
ammonia solution to get acarbose, ~~and concentrating the acarbose and which is~~
concentrated and precipitated using ethyl alcohol to get a precipitation in which
the purity of acarbose is 75~80%.

15 ~~The Adequate~~ distilled water is added to the impure acarbose powders ~~add~~
~~adequate distilled water to~~ adjust the pH value to between five and nine, ~~and~~
~~passes which is then passed~~ through a column containing AMBERJET 4400 OH
resin and α -amylglucosidase. Firstly, the column is washed with distilled water
having one to four times the volume as column and ~~solves a temperature of~~

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55~75°C ~~distilled water~~. Then, ~~collecting the acarbose concentrates are collected, and uses the ethyl alcohol is used to obtain to get a sediment~~. The sediment is then cooled and dried to get the increase the purity of the acarbose up to 95% acarbose.

5

Example 1:

~~Refer~~ Referring to Fig. 2, the present invention comprises the following steps of:

10 Step 100, ~~eliminating myselium~~; mycelium is eliminated from acarbose-containing fermentation broth by ~~centrifugating~~ centrifugation or filtering filtration;

Step 102, ~~filtrate~~ concentrating filtrate or an upper liquid (1000 ml) of the centrifuged acarbose-containing fermentation broth ~~to be consistency are concentrated by a eoneenteration concentrating system~~;

15 Step 104, ~~adding~~; adequate ethyl alcohol is added to the consistency concentrate and blending to be blended into a solution;

Step 106, ~~taking~~; an upper liquid is taken from the solution by centrifugation after blending for 30 minutes by centrifugating;

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Step 108, ~~concentrating~~; the upper liquid of the centrifuged solution is further concentrated to be a consistency by the concentrating system;

Step 110, ~~taking~~; the consistency concentrate is taken into a 99.9% ethyl alcohol solution, which wherein the amount of ethyl alcohol is ~~also~~ equal to
5 nine times the volume as the consistency of the concentrate, to get obtain a consistency liquid;

Step 112, ~~taking~~; sediment is removed from the consistency liquid by centrifuging centrifugation and solving the sediment is dissolved by water to get obtain an impure acarbose solution;

10 Step 114, ~~getting~~; using High Performance Liquid Chromatography (HPLC) to obtain an the impure acarbose solution being with 10%, 1560 mg purity and being 1560 mg by HPLC;

Step 116, ~~blending~~ a ~~strongly cation~~ strong cation exchange resin, such
as AMBERJET 1200 H resin (Rohm and Hass Company), with the acarbose
15 solution for 10 minutes, to get obtain a resin;

Step 118, ~~using~~ a 1.0N sodium chloride solution to eliminate an impurity in the resin;

Step 120, ~~using~~ a 0.75N ammonia solution to eliminate ~~a rest of the~~
further impurities impurity in the resin; and

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Step 122, ~~eluting the resin with a 1.5N ammonia solution to obtain get a~~
~~high-pure~~highly pure acarbose, in which the purity of the acarbose is 60%, 1220
mg.

5 **Example 2:**

~~Refer~~Referring to Fig. 3, the present invention comprises the following
steps ~~of~~:

Step 200, ~~adjusting the pH value of an impure acarbose to between six~~
~~and seven of an impure acarbose;~~

10 Step 202, ~~adding an a~~adding a cation exchange resin containing 250 mg sugars/g
~~resin into the impure acarbose, in~~ which the resin is AMBERJET 1200 Na resin
(Rohm and Hass Company), ~~into the impure acarbose in order to get obtain a~~
solution;

15 Step 204, ~~blending the solution for 10 minutes and taking an the upper~~
liquid;

Step 206, ~~adding a strong cation exchange resin containing 80 mg~~
~~sugars/mL into the upper liquid, in~~ which the resin is AMBERJET 1200 H resin
(Rohm and Hass Company) ~~into the upper liquid, to get obtain a~~ mixing
solution;

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Step 208, ~~;~~ mixing and shaking the mixing solution for 10 minutes to make the strong cation exchange resin ~~absorbing~~ absorb acarbose;

Step 210, ~~;~~ using a 1.0N sodium chloride solution to eliminate an impurity in the acarbose; and

5 Step 212, ~~;~~ using an ammonia solution to eliminate ~~the rest of an impurity~~ further impurities in the acarbose to ~~get obtain a high pure~~ highly pure acarbose, ~~which the~~ having a purity of acarbose is 78%, 1100 mg.

Example 3:

10 ~~Refer~~ Referring to Fig. 4, the present invention comprises the following steps ~~of~~:

Step 300, ~~;~~ adjusting the pH value ~~between six and seven~~ of an upper liquid from an impure acarbose ~~mixing~~ mixed with a strong cation exchange resin to between six and seven;

15 Step 302, ~~;~~ passing the upper liquid through a strong cation exchange resin column, 8×50 cm, containing AMBERJET 1200 H resin (Rohm and Hass Company) and washing the strong cation exchange resin in the column ~~by~~ with deionized water ~~till~~ until the absorbance of the strong cation exchange resin ~~being is~~ zero or steady;

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Step 304, ~~getting an acarbose-containing fractions fragments~~ by using ~~gradient~~ a gradiated 0.5~1.5N ammonia solution to ~~solve~~ dissolve the strong cation exchange resin;

Step 306, ~~concentrating the acarbose to be a certain volume by a~~
5 ~~concentration concentrating system~~; and

Step 308, ~~using alcohol for precipitation to precipitate the acarbose to~~
~~get obtain a high pure~~ highly pure acarbose, in which the purity of the acarbose is up 85%, 920 mg.

10 **Example 4:**

~~Refer~~ Referring to Fig. 5, the purity of the acarbose powder of the present invention is 85% ~~from as discussed in Example 3 and uses in using this example,~~
comprising the present invention comprises the steps of:

Step 402, ~~solving~~ dissolving a powder of acarbose having a, ~~which the~~
15 purity is of 83%~87%, by using distilled water, to be create a solution;

Step 404, ~~adjusting the pH value of the solution to between six and~~
~~seven of the solution~~;

Step 406, ~~passing, with a flow velocity of 1.5mL/min, the solution~~
through an α -amylglucosidase column, 8×30 cm, containing AMBERJET 4400

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OH_(Rohm and Hass company) and α -amyloglucosidase, and washing the α -amyloglucosidase column by using a volume of twice times deionized water volume twice that of as the α -amyloglucosidase column or the absorbance being 210 nm and steady;

5 Step 408, ~~;~~ eluting an acarbose from the α -amyloglucosidase column by using 65°C distilled water;

Step 410, ~~;~~ concentrating the acarbose-containing ~~fractions~~ fragments to be a volume by a ~~concentration~~ concentrating system; and

10 Step 412, ~~;~~ using alcohol ~~for precipitation~~ to precipitate the impure acarbose to get a ~~high pure~~ highly pure acarbose, ~~which the~~ having a purity of ~~the acarbose is~~ 95%, 900mg.

Advantages of the Invention

15 The above four examples ~~could get high pure~~ can achieve highly pure acarbose ~~to be appropriate for use as a medical drug, and~~ simplify the processes and decrease product costs by using ~~use low-cost resin to decrease the product~~ costs.

Therefore, ~~the foregoing is considered as illustrative only of the principles of the invention. Further, since numerous modifications and changes~~

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5 ~~will readily occur to those skilled in the art, it is not desired to limit the invention to the exact construction and operation shown and described, and accordingly, all suitable modifications and equivalents may be resorted to, falling within the scope of the invention.~~ The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.